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Early changes in lapine menisci during osteoarthritis development Part II: Molecular alterations

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Summary

Objective: Osteoarthritis (OA) is the most common form of arthritis and patients with meniscal and ligament injuries of the knee are at high risk to develop the disease. The purpose of this study was to evaluate changes occurring in both medial and lateral menisci from the knees of anterior cruciate ligament (ACL) transected rabbits at 3 and 8 weeks post-surgery. This study describes both molecular and cellular alterations in menisci during the early stages of OA development.

Design: Rabbit meniscal tissues were processed for molecular analysis: DNA and RNA concentrations were assessed, as well as semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for a subset of relevant molecules was performed. *In situ* DNA fragmentation was evaluated using the TUNEL assay.

Results: Total RNA yields from the medial meniscus were significantly elevated at both 3 and 8 weeks post-ACL transection, while in the lateral meniscus total RNA levels were unchanged following ACL transection. DNA concentrations were significantly decreased in the medial menisci only at 8 weeks post-ACL transection. Following ACL transection, analysis of *in situ* DNA fragmentation using the TUNEL assay demonstrated an increase in the number of apoptotic cells in the medial meniscus only, in particular at 3 weeks post-ACL transection, a finding which correlates with declines in DNA content. Analysis of specific mRNA levels by RT-PCR demonstrated complex changes in both menisci following ACL transection. At 3 and 8 weeks post-ACL transection, in both medial and lateral menisci, mRNA levels for type I collagen and TIMP-1 were significantly increased, while mRNA levels for decorin, TNF-α and IGF-2 were significantly depressed. In the medial meniscus, significant increases in mRNA levels for type II collagen, biglycan as well as iNOS and PAI-1 were detected at both time periods, while mRNA levels for aggrecan, type III collagen and COX-2 were significantly elevated at 3 weeks post-ACL transection and mRNA levels for MMP-1 were significantly elevated at 8 weeks post-ACL transection. In contrast, mRNA levels for COL2 and aggrecan were unchanged in the lateral meniscus following ACL transection. In the lateral meniscus, at 3 weeks post-ACL transection, type III collagen mRNA levels were dramatically increased while fibromodulin mRNA levels were significantly depressed. In the lateral meniscus, significant increases in mRNA levels for biglycan were detected at 8 weeks post-ACL transection.

Conclusion: These results show that after ACL transection complex molecular changes, as well as apoptosis, occur early, particularly in the medial meniscus. © 2001 OsteoArthritis Research Society International

Key words: Medial and lateral menisci, Molecular biology of menisci, Apoptosis, Experimental osteoarthritis.

Introduction

Meniscus injury is one of the causes of secondary osteoarthritis (OA).[1,2] To date, there have been no reports of molecular changes within menisci during the early stages of OA development. In a concurrent study, histological changes occuring in both medial and lateral menisci following ACL transection in the rabbit model of OA have been described.[3] Overt cellular and matrix alterations were found within the medial meniscus only. Formation of intrameniscal tears was detected at 3 weeks post-ACL transection and by 8 weeks post-ACL transection every medial meniscus presented with a bucket-handle tear. Histologic examination demonstrated extensive changes in collagen fiber organization as well as the coexistence of acellular areas and large cell clusters located in the outer ridge of the tear. No significant histologic changes were detected in the lateral meniscus following ACL transection. In addition, significant increase in staining for type I and III collagens was demonstrated in both menisci while type II collagen staining was increased only in the medial meniscus.

Based on our previous study, we hypothesized that the cellular and matrix changes of the menisci during the early stages of OA development would be reflected by alterations in cellular activities. Therefore, molecular changes in the rabbit meniscal tissue after ACL transection were examined using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). The pattern of mRNA expression for a subset of potentially relevant molecules has been assessed on RNA isolated from the medial and lateral menisci of non-operated rabbits (age-matched controls),

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and ACL transected rabbits at 3 and 8 weeks post-surgery. The panel of molecules to be assessed was determined based on either their known role in maintaining meniscus function (matrix molecules, proteinases and proteinase inhibitors) or their ability to influence matrix molecule and/or proteinase or proteinase inhibitor expression [growth factors and enzymes whose products have potent regulatory functions (COX-2 and iNOS)]. While not all inclusive, the chosen molecules are representative of cellular activities known to influence connective tissue function. The results indicate that complex molecular changes in the pattern of mRNA expression for both the medial and the lateral menisci occur early following ACL transection. In addition, apoptotic events occurred that were meniscus-specific and time-dependent.

Materials and methods

EXPERIMENTAL OA

Skeletally mature (12 months of age) female New Zealand White rabbits (Reimans Fur Ranch; St. Agatha, ON) were used in the present study. All rabbits had surgery under sterile conditions using a general inhalational anesthetic (1% halothane and oxygen at 1 l/min). The surgical procedure has been previously described.[3] Animals were housed in the Animal Resource Center (Faculty of Medicine) in accordance with Canadian Council on Animal Care Guidelines and with the approval of the Animal Care Committee of the Faculty of Medicine.

TISSUE PREPARATION

All animals were sacrificed by Euthanyl overdose (MTC Pharmaceuticals, Cambridge, ON) intravenously through the lateral ear vein. The medial and lateral menisci were carefully removed aseptically from the knee joint to avoid capsular contamination of the tissue. Samples were then stored at −80°C prior to processing. For TUNEL assay, tissue sections from paraffin samples were prepared as described previously.[3]

RNA EXTRACTION

Total RNA from both menisci was extracted using the TRIspin method as described previously.[4] Total RNA was quantified using the SyBr green reagent (FMC Bio-Products; Rockland, ME) and a Perkin Elmer fluorimeter. All samples were stored at −80°C until analyzed.

REVERSE TRANSCRIPTION AND SEMI-QUANTITATIVE POLYMERASE CHAIN REACTION

Reverse transcription (RT) was carried out with 1 μg total RNA using the random primers supplied in the StrataScript (tm) RT-PCR kit (PDI BioScience, Aurora, ON). Using rabbit specific primer sets described and validated in previous studies,[5,6] aliquots of cDNA were amplified by PCR as previously described.[4,6,7] For all reported experiments, conditions were determined to be in the linear range for both the PCR amplification and the image analysis system as described previously.[5] Briefly, for each group of samples (i.e. RNA from the medial and lateral menisci from operated and non-operated rabbits) all of the samples were subjected to RT at the same time and subsequently, all samples of cDNA amplified by PCR at the same time to avoid any potential experiment to experiment variation in efficiency. Each RT sample was first assessed for GAPDH (housekeeping gene) cDNA. GAPDH mRNA levels in menisci were found to be not significantly different between controls, 3 weeks and 8 weeks post-ACL transected knees in preliminary experiments. Following 21–22 PCR cycles, previously shown to yield results in the linear range of the method,[6,8] the volumes were normalized and the PCR repeated to yield very similar GAPDH integrated density values. Once the GAPDH values were determined to be similar and in the linear range of detection, the same volumes of each sample were then used to assess the cDNA levels for the remaining molecules of interest. Such an experimental construct allowed for comparisons between groups (i.e. operated vs. non-operated). For each primer set the optimal cycle number was determined and the resulting amplified bands analyzed by densitometry. The no-RT controls were negative for each primer set utilized, indicating genomic DNA contamination was undetectable. Integrated density values for the genes in question were normalized to the GAPDH values to yield a semi-quantitative assessment. Two independently isolated clones of each amplified cDNA fragment have been sequenced to verify the identity of the cDNA product.

DNA CONTENT ANALYSIS

Using the method reported by Lipman,[9] the DNA content was measured using a fluorophotometric assay as previously described.[10]

IN SITU DETECTION OF DNA FRAGMENTATION

For histochemical detection of DNA strand-breaks, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) was carried out using an ApopTag fluorescein *in situ* apoptosis detection kit (S7110; Intergen, Purchase, NY) according to the manufacturer's instructions. Negative control slides were prepared by substituting distilled water for TdT enzyme. Labeled nuclei were distinguished from the negative nuclei counterstained with DAPI and the percentage of nuclei that were positive for the TUNEL assay was assessed. At least 3000 nuclei per sample were counted.

STATISTICAL ANALYSIS

DNA and Total RNA values are given as mean ± standard deviation of six rabbits. Comparisons were made using one-way ANOVA and the BonFerroni test was subsequently applied as a post-test when comparing pairs of group means. Differences with *P*-values of less than 0.05 were considered significant.

Results

CONCENTRATIONS OF DNA

The mean concentrations of DNA per mg dry weight of tissue are given in Table I. The values for the lateral menisci for both operated groups did not differ from those in

Table I
DNA concentration per mg dry weight and total RNA concentration per mg wet weight of medial and lateral menisci

	Medial (*N*=6)			Lateral (*N*=6)		
	Control	3 weeks post-ACL-T	8 weeks post-ACL-T	Control	3 weeks post-ACL-T	8 weeks post-ACL-T
DNA	6.13	5.39	3.97	5.32	5.76	7.56
S.D.	0.43	0.89	0.65	0.37	1.86	1.15
P value	0.003			0.09		
(BonFerroni post-test)			(b**)			
RNA μg/mg tissue	0.233	0.358	0.52	0.24	0.32	0.395
S.D.	0.02	0.08	0.06	0.08	0.16	0.028
P value	0.0001			0.168		
(BonFerroni post-test)		(a*)	(b**)			
μg RNA/μg DNA ratio	0.038	0.066	0.131	0.045	0.056	0.052

(a): 3 weeks post-ACL-T vs. control; (b): 8 weeks post-ACL-T vs. control; *P<0.05; **P<0.01.

the control groups. However, at 8 weeks post-ACL transection, the mean value for the medial meniscus was significantly depressed to 66% of control values (P<0.01).

CONCENTRATIONS OF RNA

The mean concentrations of total RNA per mg wet weight of tissue are summarized in Table I. Total RNA levels in the medial menisci were significantly elevated to 126% and 184% of control values at 3 and 8 weeks post-ACL transection (P<0.05 and P<0.01, respectively). Total RNA levels were elevated in the lateral menisci after ACL transection, but not significantly.

The mean values for the ratio of μg RNA/μg DNA are presented in Table I. After ACL transection, the lateral menisci exhibited a slight elevation in their RNA/DNA ratio, whereas in the medial meniscus there was a dramatic increase in the RNA/DNA ratio at 8 weeks post-ACL transection (358% of control values).

RT-PCR ANALYSIS

Specific transcript levels from ACL-transected and non-operated control rabbits were analysed by semi-quantitative RT-PCR. Transcripts levels for structural matrix macromolecules (type I, II and III collagens, aggrecan, biglycan, decorin and fibromodulin), matrix metalloproteinases: MMP-1 (collagenase) and MMP-3 (stromelysin); proteinase inhibitors (TIMP-1 and PAI-1), growth factors (IGF-2 and TGF-β), the cytokine TNF-α and the enzymes COX-2 and iNOS are presented in Figs 1, 2 and 3.

Matrix macromolecules

Collagen type I mRNA levels were significantly increased at both 3 and 8 weeks post-ACL transection in both the lateral (P<0.001) and the medial menisci (P<0.05 and P<0.01, respectively). Transcripts for type II collagen were significantly elevated only in the medial meniscus, at both 3 and 8 weeks post-ACL transection (P<0.05). Type III collagen mRNA levels in the lateral meniscus of operated rabbits were dramatically increased to 1303% of control values at 3 weeks post-ACL transection (P<0.001) and to 582% of control values at 8 weeks post-ACL transection (P<0.05). In the medial meniscus, mRNA levels for type III collagen were significantly increased to 463% of control

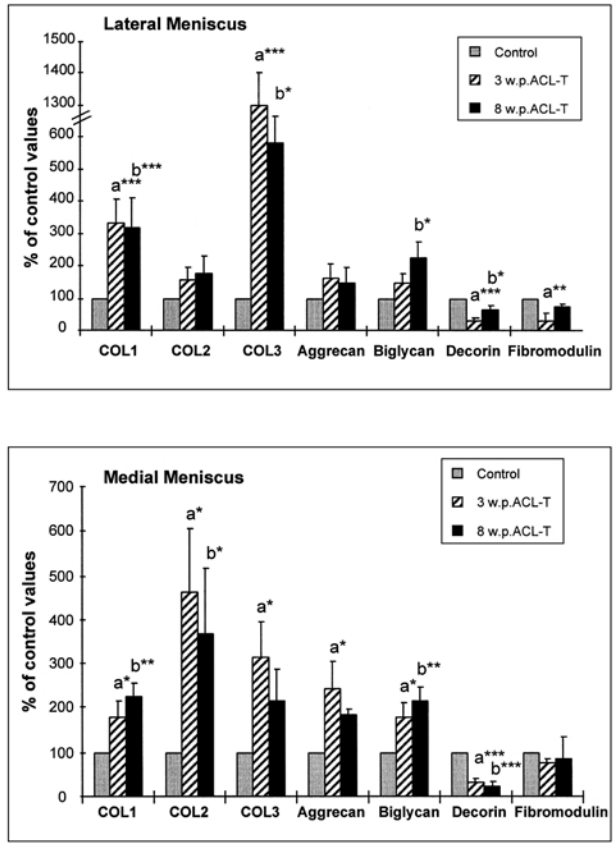

Fig. 1. Influence of ACL transection on mRNA levels for matrix macromolecules in the medial and lateral menisci. mRNA levels were determined by semi-quantitative RT-PCR as described in Materials and methods. The mean value for each molecule from the non-operated controls was set as 100% and the mean±S.E.M. values from the operated animals are presented as per cent of control values (N=6). All values indicated by an * are significantly different from control values (P<0.05). (a: 3 w. post-ACL-T vs. Control; b: 8 w. post-ACL-T vs. Control; *P<0·05; **P<0·01; ***P<0·001).

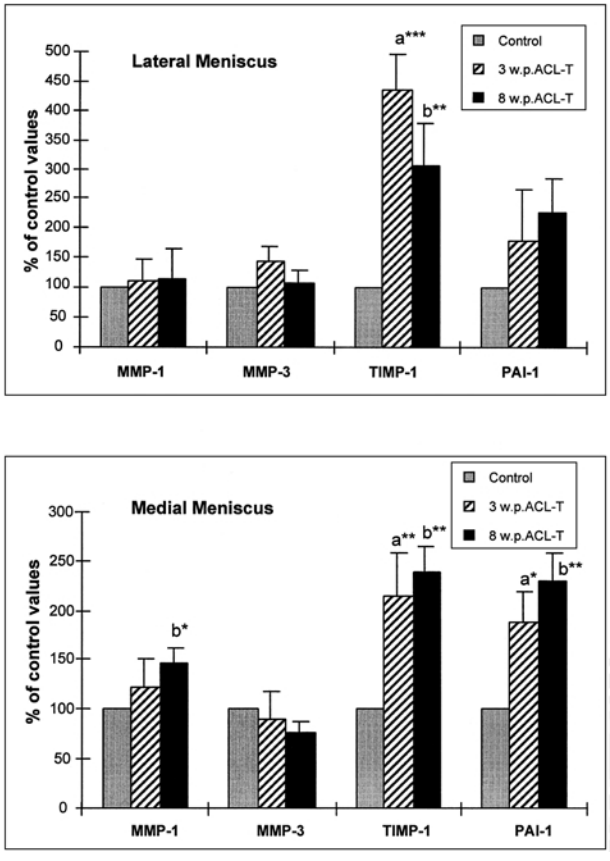

Fig. 2. Effect of ACL transection on mRNA levels for MMPs and proteinase inhibitors in the medial and lateral menisci. mRNA levels were determined by semi-quantitative RT-PCR as described in Materials and methods. The mean value for each molecule from the non-operated controls was set as 100% and the mean±S.E.M. values from the operated animals are presented as per cent of control values (N=6). All values indicated by an * are significantly different from control values (P<0.05). (a: 3 w. post-ACL-T vs. Control; b: 8 w. post-ACL-T vs. Control; *P<0·05; **P<0·01; ***P<0·001).

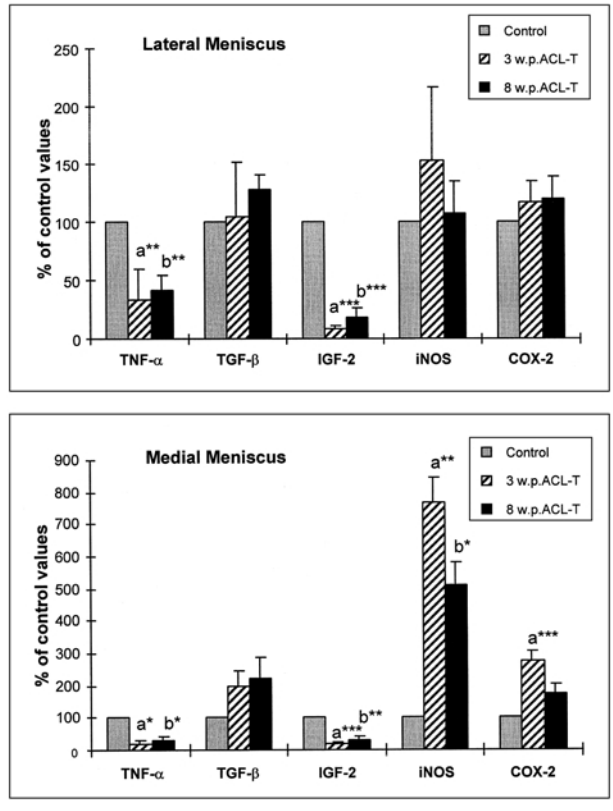

Fig. 3. Influence of ACL transection on mRNA levels for TNF-α, growth factors, iNOS, and COX-2 in the medial and lateral menisci. mRNA levels were determined by semi-quantitative RT-PCR as described in Materials and methods. The mean value for each molecule from the nonoperated controls was set as 100% and the mean±S.E.M. values from the operated animals are presented as per cent of control values (N=6). All values indicated by an * are significantly different from control values (P<0.05). (a: 3 w. post-ACL-T vs. Control; b: 8 w. post-ACL-T vs. Control; *P<0·05; **P<0·01; ***P<0·001).

values at 3 weeks post-ACL transection only (P<0.05). Aggrecan mRNA levels were significantly increased in the medial meniscus of the operated rabbits at 3 weeks post-surgery (P<0.05), but had returned to near control values at 8 weeks post-ACL transection; no changes were observed in the lateral meniscus for this molecule. Transcripts for biglycan were significantly elevated at both 3 and 8 weeks post-ACL transection in the medial menisci (P<0.05 and P<0.01, respectively), and only at 8 weeks post-ACL transection in the lateral menisci (P<0.05). In contrast, mRNA levels for decorin in both the medial and lateral menisci were dramatically depressed to 31% and 30% of control values, respectively, at 3 weeks post-ACL transection (P<0.001), and to 21% and 62% of control values, respectively, at 8 weeks post ACL transection (P<0.001 and P<0.05, respectively). In addition, fibromodulin mRNA levels were significantly depressed in the lateral meniscus at 3 weeks post-ACL transection only (P<0.01). The decrease in mRNA levels for fibromodulin was not significant in the medial meniscus at either time period.

Proteinases and inhibitors

MMP-1 (collagenase) mRNA levels were significantly elevated in the medial meniscus of operated rabbits only at 8 weeks post-ACL transection (P<0.05). In contrast, levels were unchanged in the lateral meniscus after ACL transection. Transcript levels for MMP-3 (stromelysin) were not modified after ACL transection in either the medial and the lateral menisci. mRNA levels for TIMP-1 were significantly elevated to 435% and 305% of control values in the lateral meniscus at 3 and 8 weeks post-ACL transection, respectively (P<0.001 and P<0.01, respectively). In the medial meniscus, mRNA levels for TIMP-1 were also elevated at 3 and 8 weeks post-ACL transection (P<0.01). PAI-1 mRNA levels were unchanged in the lateral meniscus following ACL transection, while PAI-1 mRNA levels were significantly increased in the medial meniscus at both 3 and 8 weeks post-ACL transection (P<0.05 and P<0.01, respectively).

Growth factors

Transcript levels for TGF-β were unchanged after ACL transection in both the lateral and medial menisci at both periods of sacrifice. In contrast, dramatic decreases in the

levels of IGF-2 were observed at both 3 and 8 weeks post-ACL transection in both the medial ($P<0.001$ and $P<0.01$, respectively) and the lateral meniscus ($P<0.001$) of operated rabbits.

Cytokine

Transcript levels for TNF-α were dramatically depressed at both 3 and 8 weeks post-ACL transection in both the medial meniscus ($P<0.01$), and the lateral meniscus ($P<0.05$) of operated rabbits.

COX-2 and iNOS

COX-2 mRNA levels were not modified after ACL transection in the lateral meniscus. In contrast, mRNA levels for COX-2 were significantly elevated to 276% of control values in the medial meniscus at 3 weeks post-ACL transection only ($P<0.001$). Similarly iNOS mRNA levels were unchanged after ACL transection in the lateral meniscus and mRNA levels for iNOS were dramatically elevated to 766% and 514% of control values in the medial meniscus of operated rabbits at both 3 and 8 weeks post-ACL transection ($P<0.01$ and $P<0.05$, respectively).

IN SITU DETECTION OF DNA FRAGMENTATION

To determine whether apoptosis plays a role in the progressive decline in the DNA concentration of the medial menisci following ACL transection, *in situ* DNA fragmentation of both medial and lateral menisci at 3 and 8 weeks post-ACL transection was assessed. Control lateral and medial menisci displayed no TUNEL-positive nuclei. In contrast, at 3 weeks post-ACL transection, medial menisci exhibited a characteristic increase in the number of TUNEL-positive nuclei in the peripheral aspect of the tear forming in the meniscus substance corresponding to the previously described proteoglycan-rich and cellular region[3] (28% of the nuclei were positive for TUNEL assay) [Fig. 4(A)]. Only a few TUNEL-positive nuclei were observed in the remaining meniscal tissue (0.1% of the nuclei were positive for TUNEL assay). At 8 weeks post-ACL transection, the medial menisci exhibited significantly fewer TUNEL-positive nuclei (0.01% of the nuclei were positive for TUNEL assay) which were randomly dispersed throughout the meniscal substance but never located within cell clusters [Fig. 4(B)]. Lateral menisci displayed no TUNEL-positive nuclei at either 3 and 8 weeks post-ACL transection.

Discussion

This report demonstrates that the transection of the ACL leads to complex changes in rabbit knee menisci at the molecular and cellular levels, and that the observed changes are both meniscus-specific and time-dependent. These findings extend and support previous observations indicating that histologic changes occur primarily in the medial meniscus following ACL transection, and begin to establish a molecular basis for the observed histologic alterations.

While no variations in total RNA concentration were found in the lateral meniscus at either of the two time periods following surgery, total RNA concentration in the medial meniscus was significantly increased at both 3 and 8 weeks-post-ACL transection. Interestingly, when normalized to DNA concentrations, the medial menisci of 3 and 8 weeks ACL transected rabbits contained 138% and 358%, respectively, of the synthetic machinery of controls in terms of total RNA (mRNA+tRNA+rRNA). This suggests that the cells of the rabbit medial meniscus are hypermetabolic after joint injury due to ACL transection. This is consistent with the report from Stockwell and Billingham that indicated meniscal cells acquire abundant golgi and endoplasmic reticulum consistent with a hypermetabolic state.[11] This anabolic activation appears to be characteristic of the medial meniscus, since no significant variations in either DNA or total RNA concentrations were found in the lateral meniscus following ACL transection. Sample to sample variation in the lateral menisci (Table I) may have obscured changes in this tissue, particularly at the 3 week time point.

The present report also demonstrated complex mRNA changes in both medial and lateral menisci following ACL transection in this lapine model of OA. In both medial and lateral menisci, mRNA levels for the major matrix macromolecules, collagen types I and III, as well as the small dermatan sulfate PG biglycan, were significantly increased, while those for the small dermatan sulfate PG decorin were significantly reduced. These findings suggest that although gross pathological as well as histological changes were relatively moderate in the lateral meniscus compared with the medial meniscus,[3] molecular changes occur at the mRNA level in the lateral meniscus in response to joint injury following ACL transection.

The relative proportion of the collagens and the proteoglycans in menisci determines physical properties, and any changes in these proportions may influence tissue behavior. Interestingly, following ACL transection, the cells of the medial meniscus exhibited a pattern of mRNA expression that corresponds to a more fibrochondrocytic phenotype, with significantly increased mRNA levels not only for type I and III collagens, but also for type II collagen and aggrecan (at least at 3 weeks post-ACL transection for the latter). The discoordinate expression of type II collagen and aggrecan mRNA levels observed at 8 weeks post-ACL transection is consistent with the findings previously reported by Matyas *et al.* in the canine model of OA.[12,13] In contrast, the lateral meniscus exhibited a pattern of mRNA expression that correspond more to a fibroblastic phenotype, with highly significant increases in mRNA levels for collagens type I and III only and significantly depressed mRNA levels for fibromodulin (at least at 3 weeks post-ACL transection). The former is highly significant considering that normal mRNA levels for type I and III collagens are significantly higher in the medial meniscus compared with the lateral meniscus in skeletally mature rabbits.[10] Interestingly, fibromodulin is also a small keratan sulfate PG that has been shown to inhibit formation of fibrils in a solution of type I collagen.[14] Moreover, fibromodulin has been described to be present in highest amounts in the inner third of porcine menisci, corresponding to the hyaline-like cartilage region of the porcine menisci.[14] Very interestingly, these findings for the collagens at the molecular level correlate with our previously reported increase in staining for type I and III collagens in the lateral menisci and for type I, II and III collagens in the medial menisci.[3] Such findings support the conclusion that meniscus-specific cellular responses at the mRNA level occur following ACL transection.

Transection of the ACL did not lead to any detectable changes in mRNA levels for MMP-3 in either the medial or the lateral meniscus. In contrast, while mRNA levels for

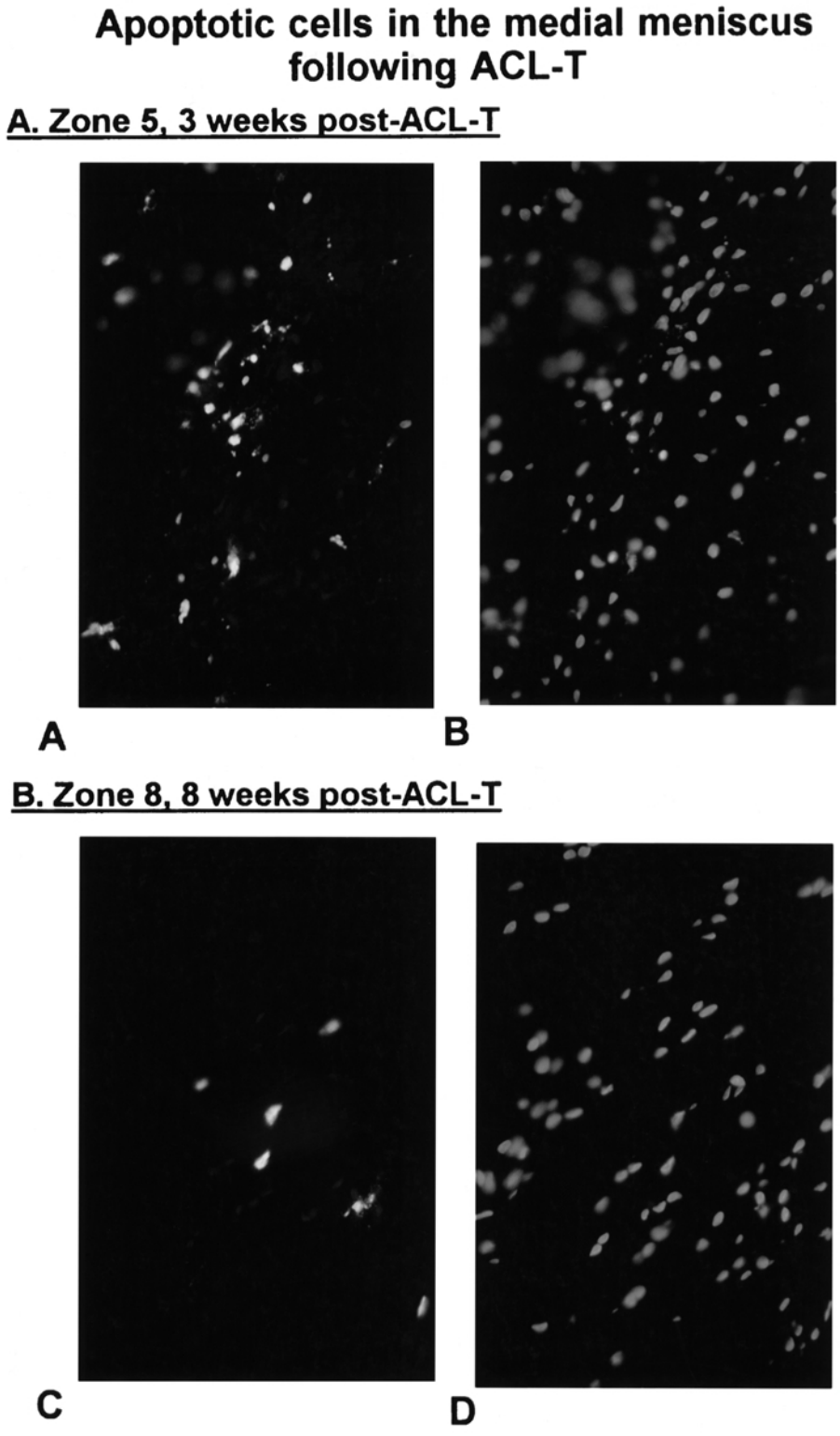

Fig. 4. Apoptotic nuclei in the medial meniscus. Micrographs of sections of medial meniscus at 3 and 8 weeks post-ACL transection. *In situ* analysis of DNA fragmentation detected by fluorescein-conjugated nucleotide labeling of 3′-OH DNA ends. Labeled nuclei were visualized under fluorescence for TUNEL-positive nuclei. At 3 weeks post-ACL transection, labeled nuclei were mostly located in the meniscal outer region with a few TUNEL-positive nuclei within the remaining meniscal tissue (Panel A). At 8 weeks post ACL transection, fewer labeled nuclei were randomly dispersed throughout the meniscal tissue (Panel C). Panels B and D represent the DAPI counterstained nuclei corresponding to the corresponding sections (Panels A and C, respectively).

MMP-1 were unchanged in the lateral meniscus following ACL transection, a significant increase in MMP-1 transcript levels was observed at 8 weeks post-ACL transection in the medial meniscus. The more severe morphological alterations observed in the medial meniscus could be the result of enzymatic degradation of the extracellular matrix, possibly through the action of MMP-1 or other MMPs not assessed. Only two MMPs and two proteinase inhibitors were assessed at the mRNA level in the present study. Other potential MMPs, such as the collagenases MMP-13 and MMP-8, could be implicated in the degradation of the meniscal matrix, as it has been described in osteoarthritic articular cartilage.[15,16] Moreover, one must keep in mind that the activity of the MMPs is regulated not only at the transcriptional level, but also at the translational and post-translational levels (activation of the enzyme and inhibition through various proteinase inhibitors) and only mRNA levels were assessed in this study.

The dramatic decreases in mRNA levels for TNF-α observed in both menisci suggest this cytokine does not play a major role in matrix alterations in early OA. In contrast, COX-2 mRNA levels were signifcantly increased only in the medial meniscus. COX-2 is an inducible enzyme whose products (i.e. prostaglandins) have been shown to have potent regulatory functions on cells.[17] Increased levels of prostaglandin E products of COX-2 could also influence the proteinase-proteinase inhibitor balance in the tissue.[18] The involvement of COX-2 in the changes occuring in the medial menisci could be defined in the future through the use of COX-2 specific inhibitors.

The DNA concentration in the medial meniscus was significantly reduced by 34% at 8 weeks post-ACL transection. In contrast, no variations in DNA concentration were found in the lateral meniscus. Considering the large acellular areas described in the medial menisci at 8 weeks post-ACL transection,[3] the significant reduction in DNA content within the medial meniscus at 8 weeks post-ACL transection probably reflects cell loss. This conclusion was supported by the analysis of *in situ* DNA fragmentation in menisci by the TUNEL assay. While no evidence for *in situ* apoptosis was detectable in either control menisci or lateral menisci from ACL transected knees, a significant increase in the number of apoptotic nuclei was detected in medial menisci at 3 and 8 weeks post-ACL transection. At 3 weeks post-ACL transection, some of the apoptotic nuclei were located throughout the meniscal tissue, but the large majority were located in the previously described proteoglycan-rich and cellular region located on the outer side of the fissure.[3] Only a few apoptotic nuclei were observed throughout the meniscal tissue at 8 weeks post-ACL transection. In contrast to the recent study of Hashimoto *et al.*,[19] in which the presence of apoptotic cells was reported within rabbit meniscal cell clusters at 9 weeks following ACL transection, in the present study apoptotic nuclei were never located within the cell clusters that were detected along the edges of the bucket-handle tear. The time course of the detection of these apoptotic cell probably explains the changes in DNA content. The occurrence of apoptotic nuclei and the mRNA levels for the enzyme iNOS also may be related. iNOS mRNA levels were dramatically increased only in the medial meniscus, following ACL transection. Assuming the increases in iNOS mRNA levels are reflected by elevated protein activity, the dramatic increase in iNOS mRNA levels in the medial meniscus could be related to the observed elevation in apoptotic nuclei and subsequent decrease in DNA concentration in this tissue. The present results also support the report of Hashimoto *et al.*, describing nitric oxide production and apoptosis in cells of the meniscus in this rabbit model of OA.[19] In addition, it is probably relevant that mRNA levels for IGF-2 were dramatically depressed following ACL transection in the present study. In several animal models, IGF-2 expression by pancreatic islet cells, as well as neuroblastoma cells, has been shown to be associated with a reduction in both NO production and apoptotic events.[20–22] Moreover, similar observations have been reported with equine fetal and neonatal chondrocytes *in vitro*.[23] Therefore, in the medial meniscus IGF2 suppression, as well as increased iNOS mRNA levels, could pre-dispose to development and progression of apoptosis. However, the IGF2 mRNA suppression in the lateral meniscus does not appear to be related to apoptotic events.

In conclusion, the present study demonstrates that during the early phases of joint responses to ACL transection complex mRNA changes occur in the menisci that are consistent with structural changes. Changes in mRNA levels for type I, II and III collagens correlated with previous detection of increased collagen staining in the menisci following ACL transection.[3] The decrease in DNA content in the medial meniscus at 8 weeks post-ACL transection correlated with the detection of apoptotic events, and iNOS and IGF2 mRNA changes. Based on the significant medial meniscus-specific increases in mRNA levels for iNOS and COX-2, future studies will determine whether therapeutic interventions such as iNOS and/ or COX-2 inhibitors can alter disease progression in this model system.

Acknowledgments

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Abbreviations

ACL, anterior cruciate ligament; bFGF, basic fibroblast growth factor; COL1, type I collagen gene; COL2, type II collagen gene; COL3, type III collagen gene; COX-2, cyclooxygenase-2; DAPI, 4′,6 diamino-2-phenylindole; GAG, glycosaminoglycan; GAPDH, glyceraldehyde phosphate dehydrogenase; IGF-2, insulin-like growth factor-2; iNOS, inducible nitric oxide synthase; MMP-1, matrix metalloprotease-1; MMP-3, matrix metalloprotease-3; OA, osteoarthritis; PAI-1, plasminogen activator inhibitor-1; PG, proteoglycan; RT-PCR, reverse transcriptase-polymerase chain reaction; TGF-β, transforming growth factor-β; TIMP-1, tissue inhibitor of metalloproteases-1; TNF-α, tumor necrosis factor-α.